DAILY QUALITY CONTROL (QC) OF ATTUNE NXT

1.0 Purpose

This document describes the daily quality control (QC) of the Attune Nxt Flow Cytometer, a cell analyser within the QMRI Flow Cytometry Facility. The QC uses a manufacturers defined automatic test which checks the laser alignment, laser delays and detector sensitivities to ensure optimum performance of the instrument before user sample runs.

2.0 Scope

This is the standard operating procedure (SOP) for the daily QC of the Attune Nxt and should be read and understood by all facility staff and users who have been approved by facility staff.

3.0 Responsibilities

Responsibilities for the daily QC of the Attune Nxt and the test outcomes fall to the facility staff and those users who are trained to perform the test. QC fails as determined by the automatic software should be treated within the knowledge base of the operator and escalated via the facility hierarchy then to the manufacturer, as appropriate.

4.0 Procedure

The detailed protocol for the Attune Nxt Daily QC can be found in the attached appendix.
Start-up of the Instrument is detailed within SOP_QFCF_501_Attune_StartUp.

5.0 Related Documents

Facility Risk Assessment: RA1_QFCF_General
Facility Guidelines
Safe System Of Work: SSW_QFCF_005_Attune
COSHH: COSHH_QFCF_001_General
Standard Operating Procedures: SOP_QFCF_501_Attune_Startup

6.0 Approval & Sign Off

Author:

Name: Mari Pattison

Position: Flow Cytometry Research Assistant

Signature: Date: 10th March 2020

Manager Approval:

Name: Shonna Johnston

Position: Facility Manager

Signature: S. Johnston Date: 10th March 2020

Attune Nxt_Daily QC

This QC procedure should be performed after the daily startup:
SOP_QFCF_501Appendix_Attune_StartUp

Daily Clean (Can be replaced by a Weekly Deep Clean)

- Navigate to Instrument tab and click **Deep Clean** arrow then select **Quick**. ***008**
- Follow onscreen instructions to load an empty cleaning plate and a 3ml tube of **Bleach** (10 % solution). ***009**
- Click **Next**.
- This Deep Clean cycle lasts ~15 mins.
- Once completed follow onscreen instructions to remove tube and allow Rinse to finalise.
- Remove plate from autosampler and rinse plate wells A1-A4 with dH2O and flick away into the hand basin.

QC/Performance Test (To be run after a Daily or Weekly Clean)

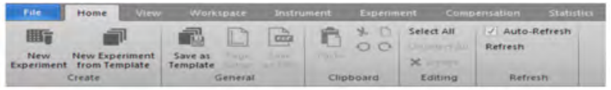
- Click Performance Test. ***013**
- Verify instrument configuration is correct by lifting the lid of the Attune. ***010**
- Vortex vial of **Attune Performance Tracking Beads**. ***011**
- Place 1 drop of Attune Performance Tracking Beads in a fresh 5 ml tube, add 2 ml of **Focusing Fluid** and vortex.
- Place on tube lifter.
- Click **Run Performance Test**.
- Raise tube lifter as prompted. ***009**
- Allow QC test to complete.
- Verify Results
    - If a **PASS**, then allow to Rinse and run a Sanitize Attune SIP (Instrument tab) following onscreen instructions. ***008 *015**
    - If a **FAIL**, then perform Sanitise SIP cleaning, recheck filters etc, follow any suggested onscreen instructions and repeat.
    - If repeatedly failing, then escalate via the facility hierarchy.

* denotes that a **How To…. Guide** can be found laminated in the Instrument Information Box and an electronic version in the **ALL USERS** folder on the workstation desktop.

Attune Nxt_008: Software, Experiment Tabs

Home Tab

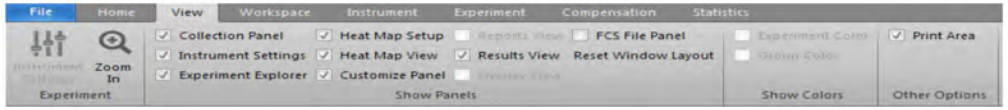
- new experiments & templates



☑ Auto-Refresh

View Tab

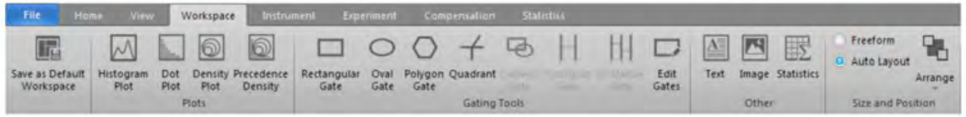
- customise software view



☑ Collection Panel
☑ Instrument Settings
☑ Experiment Explorer
☑ Heat Map Setup
☑ Heat Map View
☑ Customize Panel
☑ Results View
☐ FCS File Panel
☑ Print Area

Workspace Tab

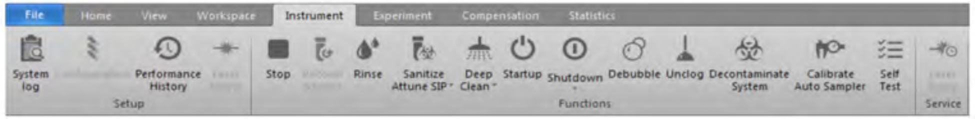
- data plots, gating, layout control



○ Freeform
● Auto Layout

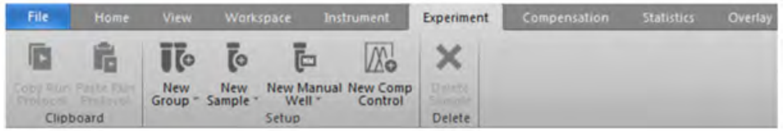
Instrument Tab

- system log, performance history, cleaning procedures



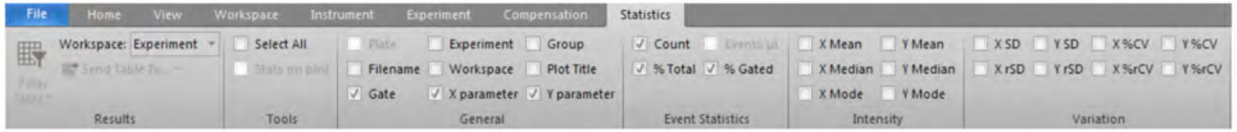
Experiment Tab

- new group/sample/well



Statistics Tab

- various statistical analyses

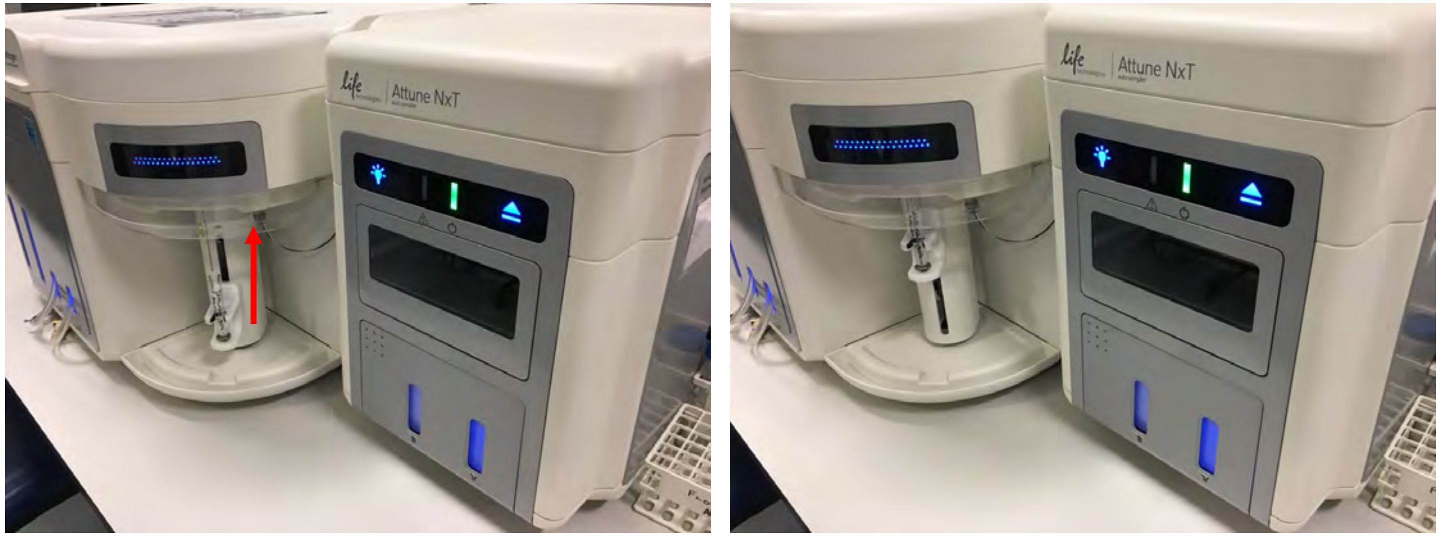


Workspace: Experiment
☐ Select All
☐ Filename
☑ Gate
☐ Experiment
☐ Workspace
☑ X parameter
☐ Group
☐ Plot Title
☑ Y parameter
☑ Count
☑ % Total
☑ % Gated
☐ X Mean
☐ X Median
☐ X Mode
☐ Y Mean
☐ Y Median
☐ Y Mode
☐ X SD
☐ X rSD
☐ Y SD
☐ Y rSD
☐ X %CV
☐ X %rCV
☐ Y %CV
☐ Y %rCV

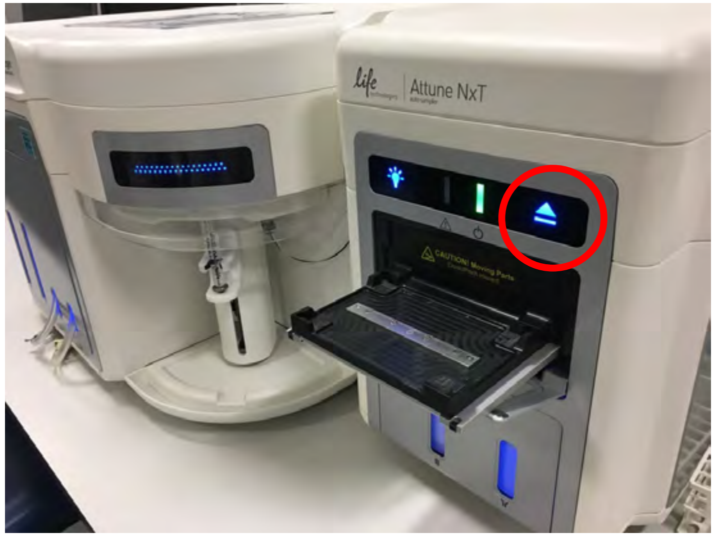
Attune Nxt_009: Sample Management

Tube placed under SIP and lifter raised upward.

To remove, pull lifter down before removing tube

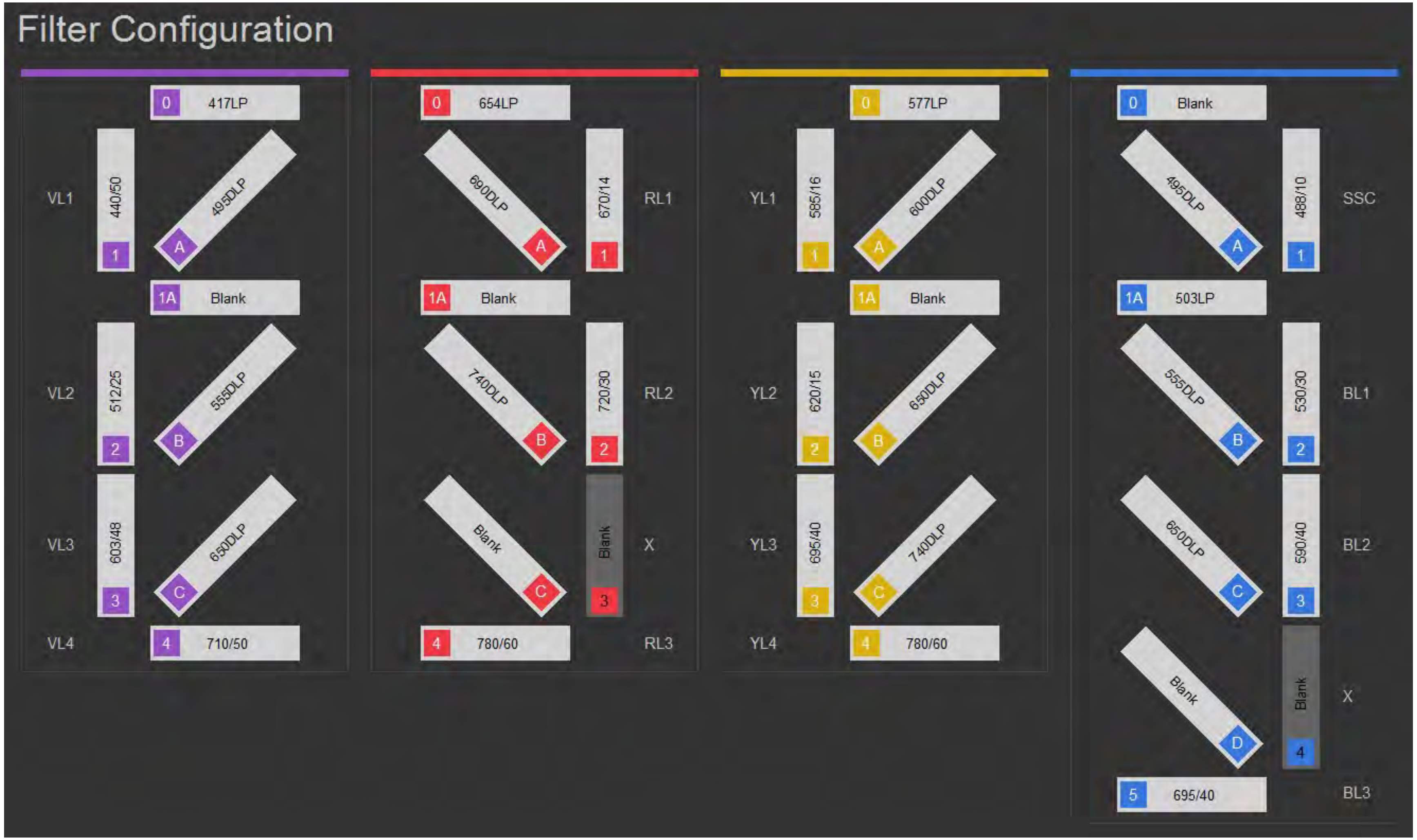


Autosampler opened by pressing furthest right button, place lidless plate on stage and press same button again to close.



Attune Nxt_010: Configurations

Used for panels containing VL1 (BV421;ef450etc) and VL2 (BV510; L/D Aqua)

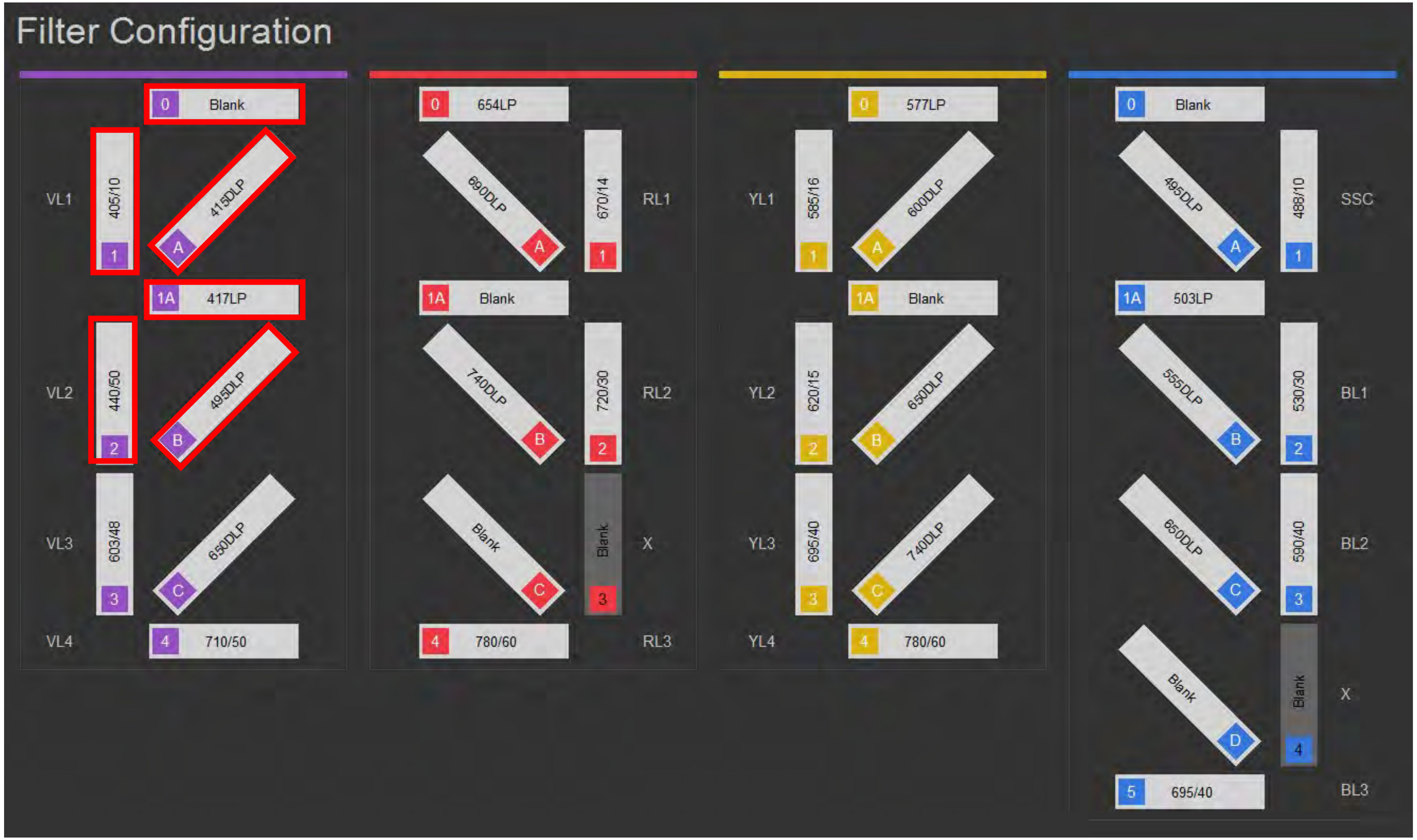


Attune Nxt_010: Configurations

Used for Panels requiring Violet Side Scatter (V-SSC) eg Bacteria, EVs and samples with high rbcs or experiments/panels using VL 1 or 2 and created before 31st August 2019

▭

Denotes filters that need changed for this configuration



Filter Configuration

0 Blank
VL1 405/10 1
A 415DLP
1A 417LP
VL2 440/50 2
B 495DLP
VL3 603/48 3
C 650DLP
VL4 4 710/50

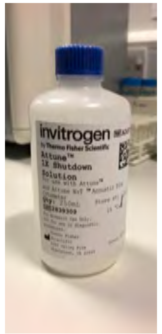
0 654LP
A 690DLP
RL1 670/14 1
1A Blank
B 740DLP
RL2 720/30 2
C Blank
X Blank 3
RL3 4 780/60

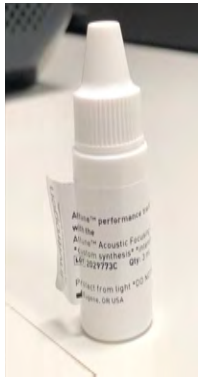
0 577LP
YL1 585/16 1
A 600DLP
1A Blank
YL2 620/15 2
B 650DLP
YL3 695/40 3
C 740DLP
YL4 4 780/60

0 Blank
A 495DLP
SSC 488/10 1
1A 503LP
B 555DLP
BL1 530/30 2
C 650DLP
BL2 590/40 3
D Blank
X Blank 4
BL3 5 695/40

QMRI Flow Cytometry
Cell Sorting facility

Attune Nxt_011: Instrument Reagents





	Focusing Fluid	**PBS**	**Bleach**	**dH_2O**	**Wash Solution**	**Shutdown Solution**	**Hellmanex III**	**Debubble Solution**	**Attune Performance Tracking Beads**
Storage	RT	RT	RT	RT	RT	RT	RT	RT	4°C
Location	Workstation: 50ml Falcon Shelf: 1L bottles Floor: 10L boxes	LSR Workstation: 50ml Falcon C2.35 sink: 5L box	C2.36: workstation 10% working solution in wash bottle C2.36: Bleach Storage Box [stock solution]	Workstation: wash bottle C2.35 sink: 1L bottle	C2.36: shelf	C2.36: shelf	C2.36: shelf	C2.36: shelf	Cold Room, Staff Reagent Box
Expiry (after opening)	50ml Falcon: 1 month Replace weekly 1L bottle: 6 months 10L box: 6 months	50ml Falcon: 1 month 5L box: 6 months	Wash Bottle: 1 month Stock Solution: 3 years	1 month for both	6 months	6 months	3 years	6 months	1 year

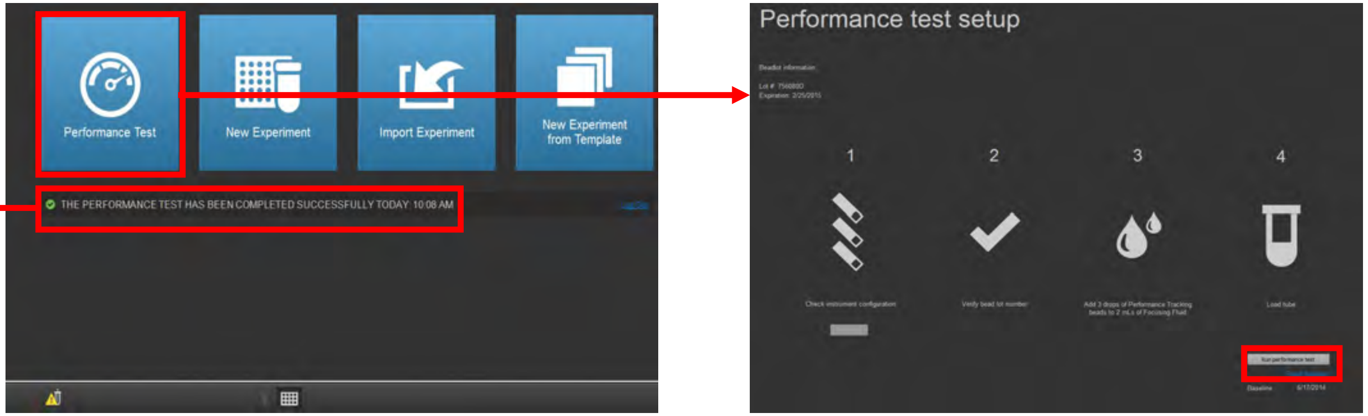
QMRI Flow Cytometry
Cell Sorting facility

Attune Nxt_013: Performance Test

✓ **Performance Test run successfully today.**
Ready to run samples

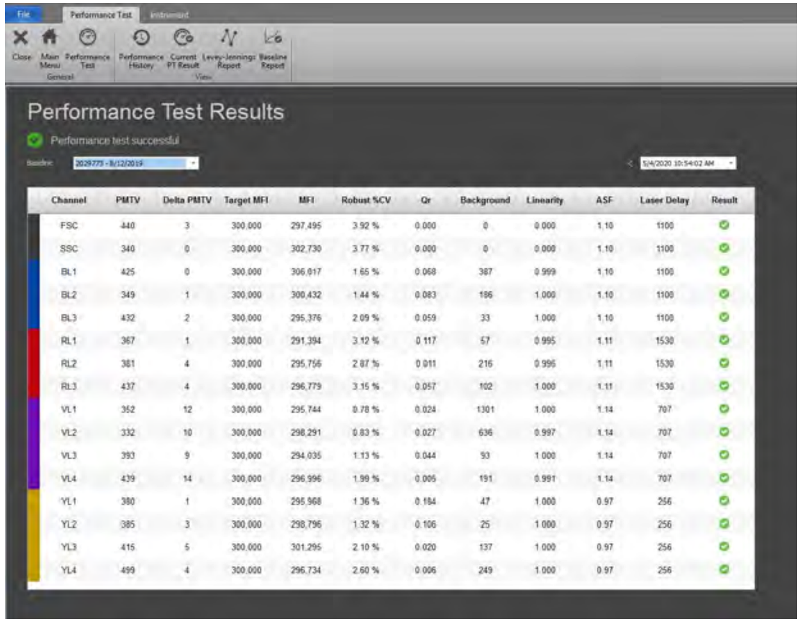
⚠ **Performance Test not completed today.**
Run Performance Test before running samples

⚠ **Performance Test not run successfully today.**
Rerun Performance Test before running samples



THE PERFORMANCE TEST HAS BEEN COMPLETED SUCCESSFULLY TODAY 10:08 AM

To run Performance Test, select left blue icon & follow instructions



Performance Test Results

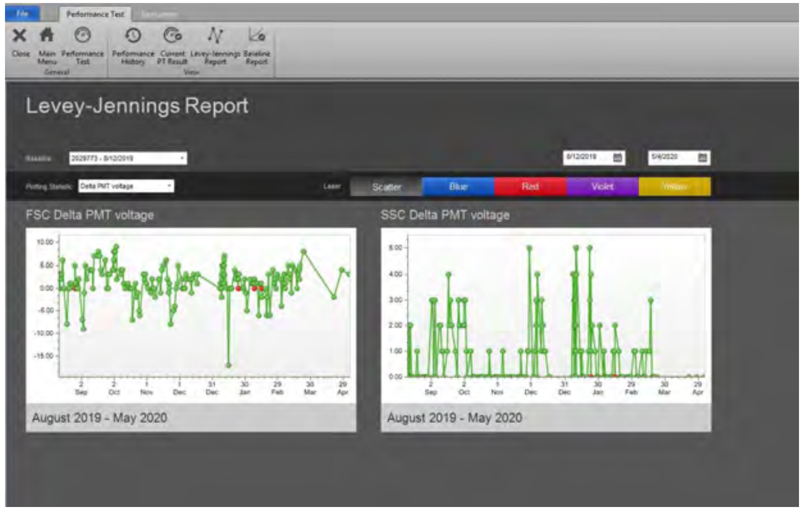
✓ Performance test successful

Baseline: 2029773 - 8/12/2019

5/4/2020 10:54:02 AM

Channel	PMTV	Delta PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	440	3	300,000	297,495	3.92 %	0.000	0	0.000	1.10	1100	✓
SSC	340	0	300,000	302,730	3.77 %	0.000	0	0.000	1.10	1100	✓
BL1	425	0	300,000	306,017	1.65 %	0.068	387	0.999	1.10	1100	✓
BL2	349	1	300,000	300,432	1.44 %	0.083	186	1.000	1.10	1100	✓
BL3	432	2	300,000	295,376	2.09 %	0.059	33	1.000	1.10	1100	✓
RL1	367	1	300,000	291,394	3.12 %	0.117	57	0.995	1.11	1530	✓
RL2	381	4	300,000	295,756	2.87 %	0.011	216	0.996	1.11	1530	✓
RL3	437	4	300,000	296,779	3.15 %	0.057	102	1.000	1.11	1530	✓
VL1	352	12	300,000	295,744	0.78 %	0.024	1301	1.000	1.14	707	✓
VL2	332	10	300,000	298,291	0.83 %	0.027	636	0.997	1.14	707	✓
VL3	393	9	300,000	294,035	1.13 %	0.044	93	1.000	1.14	707	✓
VL4	439	14	300,000	296,996	1.99 %	0.005	191	0.991	1.14	707	✓
YL1	380	1	300,000	295,968	1.36 %	0.184	47	1.000	0.97	256	✓
YL2	385	3	300,000	298,796	1.32 %	0.106	25	1.000	0.97	256	✓
YL3	415	5	300,000	301,295	2.10 %	0.020	137	1.000	0.97	256	✓
YL4	499	4	300,000	296,734	2.60 %	0.006	249	1.000	0.97	256	✓

Click Performance tracking to view change in detector voltages, CVs and laser delays.
Report any trends.



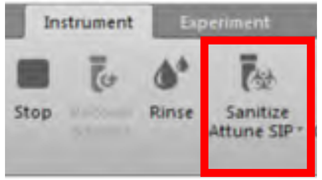
QMRI Flow Cytometry
Cell Sorting facility

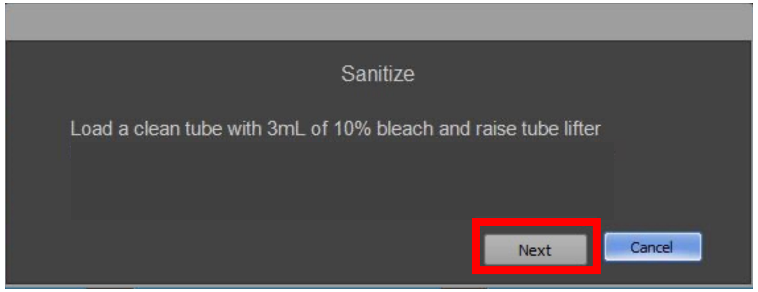
Attune_015: Cleaning

There are 3 cleaning processes that can be initiated dependent on sample type and acquisition method:

1. **Standard Samples – Autosampler Not Used**
2. **Standard Samples – Autosampler Used**
3. **Bacterial or Human/COVID-19 Samples**

1.



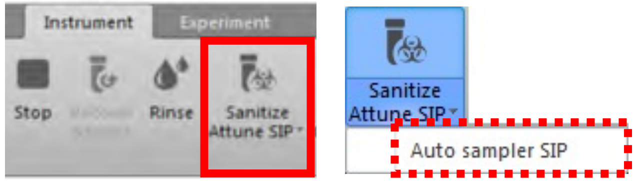

Sanitize

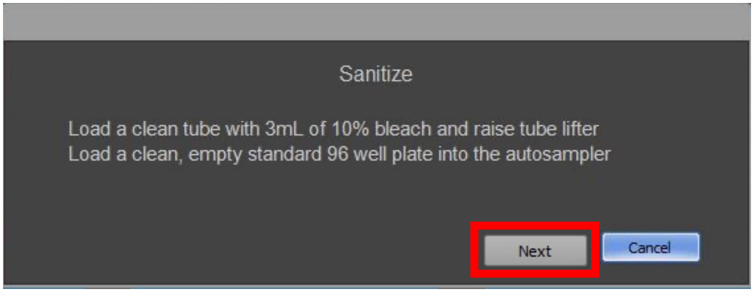
Load a clean tube with 3mL of 10% bleach and raise tube lifter

- Go to **Instrument tab** and click **Sanitise SIP** button
- Follow on screen prompt and click **Next**
- When sanitise cycle is finished remove 5ml tube
- Dispose of liquid in tube using liquid waste container and leave tube in rack
- **Log out** of PPMS

Please note that the Bleach should be ***replaced*** *at every Sanitize SIP as the 3ml of Bleach is replaced by the Focusing fluid and is therefore useless in subsequent sanitize cycles.*

2.



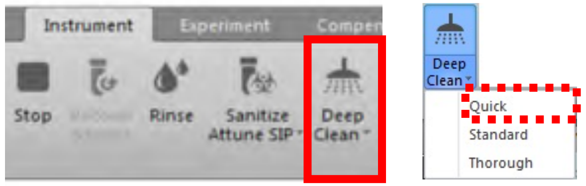

Sanitize

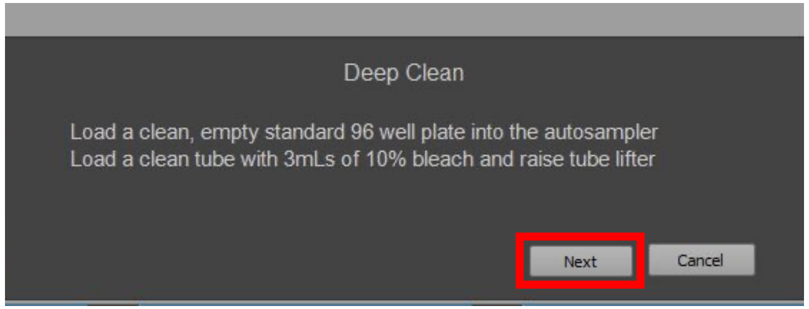
Load a clean tube with 3mL of 10% bleach and raise tube lifter
Load a clean, empty standard 96 well plate into the autosampler

- Go to **Instrument tab** and click arrow under the **Sanitise SIP** button and select **Standard**
- Follow on screen prompt and click **Next**
- When sanitise cycle is finished remove 5ml tube and cleaning plate
- Dispose of liquid in tube using liquid waste container and leave tube in rack
- Rinse wells A1-A4 with dH2O, flick into sink, replace lid and leave plate at workstation
- **Log out** of PPMS

Please note that the Bleach should be ***replaced*** *at every Sanitize SIP as the 3ml of Bleach is replaced by the Focusing fluid and is therefore useless in subsequent sanitize cycles.*

3.




Deep Clean

Load a clean, empty standard 96 well plate into the autosampler
Load a clean tube with 3mLs of 10% bleach and raise tube lifter

- Go to **Instrument tab** and click arrow under the **Deep Clean** button and select **Quick** (Cycle takes ~15minutes)
- Follow on screen prompt and click **Next**
- When deep clean cycle is finished remove 5ml tube and cleaning plate
- Dispose of liquid in tube into liquid waste container and leave tube in rack
- Rinse wells A1-A4 with dH2O, flick into sink, replace lid and leave plate at workstation
- **Spray workstation area** with **Virkon** and wipe away with white roll then repeat with **EtOH. NB.**Virkon and EtOH can found beside the sink
- **Log out** of PPMS
- **In addition,** it may be necessary to change the waste tank at this stage too

Please note that the Bleach should be ***replaced*** *at every Deep Clean as the 3ml of Bleach is replaced by the Focusing fluid and is therefore useless in subsequent sanitize cycles.*